## REMARKS

**Status of the Claims**

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-59 are pending. Claims 17, 24-28, 32, 45, 47-49, 51 have previously been cancelled.

Claim 10 is deleted herein in view of claim 11.

Claims 55-59 are added herein.

Support for new claims 55 and 58 can be found, for example, in original claim 1 and in the specification at page 20, line 22 to page 21, line 5 and Example 1.

Support for new claims 57 and 59 and can be found, for example, in original claims 9-11 and throughout the specification.

Support for new claim 56 can be found, for example, in the specification at page 12, lines 22-24.

**Rejection of Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53--35 U.S.C. 103(a)**

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, and 52-54 stand finally rejected under 35 U.S.C. 103(a) as obvious over WO 97/24447 to Song et al. (Song) in view of U.S. Patent No. 5,783,567 to Hedley et al. (Hedley) and E. Fattal et al., *Journal of Controlled Release*, 53 (1998) 137-143 (Fattal).

**Rejection of Claim 54**

Claim 54 is directed to a method of transfecting dendritic cells. The method comprises incubating dendritic cells and a transfection agent that comprises a polynucleotide (which encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor) adsorbed on surfaces of microparticles. The incubation is performed *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

For a proper obviousness rejection, the differences between the subject matter sought to be patented and the prior art must be such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains. 35 U.S.C. §103. The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been

obvious. MPEP 2141. " '[R]ejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.' " *KSR International Co.* v. *Teleflex Inc.*, 550 U.S. ___, 82 USPQ2d 1385 (2007), quoting *In re Kahn* , 441 F.3d 977, 988, (Fed. Cir. 2006). In addition, there must be a reasonable expectation of success. See MPEP 2143.02.

Song describes compositions and methods useful for stimulating an immune response against one or more disease associated antigens by genetically modifying dendritic cells *in vivo* or *ex vivo*. See Song Abstract. Gene delivery vehicles are described, which are targeted to dendritic cells, whether *in vivo* or *in vitro*, and which comprise a dendritic cell targeting element and an expression vector which directs expression of at least one disease associated antigen. *Id.* at page 2, lines 16-19. The dendritic cell targeting element can be any molecule which targets the gene delivery vehicle to a dendritic cell, for example, a high affinity binding pair, an antibody reactive against a dendritic cell surface marker, an antigen binding domain derived from an antibody reactive against a dendritic cell surface marker, or a hybrid envelope protein. *Id.* at page 3, lines 16-31.

In some embodiments, the expression vector is carried by a recombinant virus, including DNA and RNA viruses, preferably a recombinant virus derived from either a negative strand RNA virus or a positive strand RNA virus. *Id.* at page 2, lines 19-22. Various negative and positive strand viruses are set forth, for example, at page 2, line 22 to page 3, line 2. In other embodiments, the gene delivery vehicle is non-viral gene delivery vehicle. *Id.* at page 3, lines 5-7. In some of these embodiments, the expression vector is complexed with one or more polynucleotide condensing agents, including polycations. *Id.* at page 3, lines 8-10. In some of these embodiment, the expression vector is associated with lipids, preferably encapsulated in liposomes. *Id.* at page 3, lines 13-15. In other embodiments, the expression vector is complexed only with the dendritic cell targeting element. *Id.* at page 3, lines 12-13.

Thus, Song teaches that the gene delivery vehicle may be viral or non-viral, and that dendritic cells may be genetically modified *in vivo* or *ex vivo*. Song, however, clearly expresses a preference for *in vivo* (direct injection) delivery of recombinant retroviruses carrying an expression vector. *Id.* at page 27, lines 25-27. Furthermore, the non-viral vehicles taught by Song (i.e., polynucleotides associated with condensing agents or encapsulated in liposomes) are

unrelated to the delivery vehicle of claim 54 (i.e., polynucleotides adsorbed on surfaces of microparticles), other than in the sense that they are "non-viral" techniques.

Recognizing that Song is deficient, the Examiner argues that Hedley supplements Song through its teachings regarding the use of microspheres comprising biodegradable polymers and it's use of DNA plasmids to introduce and express antigens encoded by the plasmids in antigen presenting cells such as macrophages and dendritic cells, both *in vitro* and *in vivo*, for the purpose of stimulating antigen specific immune responses. See the Office Action mailed September 22, 2004, page 5. It is further argued that Hedley provides motivation for introducing plasmid DNA encoding an antigen to antigen presenting cells such as macrophages and dendritic cells using biodegradable microspheres by teaching that DNA combined with biodegradable microparticles is efficiently phagocytosed by antigen presenting cells and is an effective means for introducing nucleic acids into these cells. *Id.* at page 6. The Examiner further argues that Hedley recognizes that dendritic cells are a "legitimate target" for microparticle transfection when stating that the point of introduction of plasmid/microparticles to skin is the transfection of dendritic cells. *Id.*

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. The Examiner's view of Hedley, however, has been unduly influenced by hindsight that has been gleaned from Applicant's disclosure.

In particular, Hedley is said to be based on the discovery that microparticles containing nucleic acids and having an appropriate size for phagocytosis can be made without adversely affecting nucleic acid integrity. Hedley at col. 1, lines 32-37. Hedley states that phagocytosis of microparticles by macrophages and other antigen presenting cells (APCs) is an effective means for introducing the nucleic acid into these cells. *Id.* at col. 8, lines 13-15. Hedley teaches various means of *in vivo* delivery. *Id.* at col. 8, lines 20-34.

The microparticle-based technique of Hedley, therefore, has certain things in common with the teachings of Song. For example, Hedley describes non-viral gene delivery, while Song teaches the use of any gene delivery vehicle, whether viral or non-viral. Moreover, Hedley's composition is administered *in vivo*, whereas Song teaches the use of any mode of administration, whether *in vivo* or *ex vivo*.

However, the Examiner's argument that Hedley supplements Song through its teachings regarding the use of microparticles to introduce and express antigens in *dendritic* cells *in vitro* is clearly the result of undue hindsight.

In particular, as pointed out by the Examiner, Hedley does speculate at col. 8, lines 25-27 that, during intradermal delivery, microparticles could be *introduced to* antigen presenting cells of the skin, including dendritic cells and Langerhans cells (presumably because they are inherently present in the skin). This, however, is the only mention of dendritic cells in all of Hedley. Moreover, unlike the claimed method, this single mention of dendritic cells is in conjunction with an *in vivo* technique.

Elsewhere, Hedley refers to "macrophages and other antigen presenting cells (APCs)." See col. 8, lines 13-14. Indeed, the entire disclosure of Hedley is based upon the introduction of nucleic acids into macrophages, with the Examples describing the phagocytosis of DNA-containing polymeric microparticles by macrophages and the subsequent expression of that DNA.

One of ordinary skill in the art would read Hedley as taking advantage of macrophages' superior ability to phagocytose various materials (e.g., cells, cellular debris, etc.) in the body. However, one of ordinary skill in the art could not reasonably expect, based on the macrophage-based data of Hedley that dendritic cells would also take up the microparticles (and thus the associated nucleic acids) and express the DNA therein.

For example, one of ordinary skill in the art would immediately recognize that particulate uptake by macrophages, which are aggressively phagocytic, would not be predictive of particulate uptake by dendritic cells, which are not. In this regard, see, for example, M.F. Lipscomb et al., *Physiol Rev* 82:97-130, 2002 (attached), where it is noted that macrophages can be separated from dendritic cells by a negative step, based on the fact that macrophages are "avidly phagocytic" and thus readily phagocytose silica particles, latex beads, or carbonyl iron particles, while dendritic cells do not. See page 98, col. 2. See also E. Karhumaki et al., *Clin. Exp. Immunol.* 1993; 91: 482-488 (attached), which describes a rapid and simple technique for purifying dendritic cells from human peripheral blood. In this technique, cells were incubated with carbonyl iron particles. Subsequently, the cells that had phagocytosed the iron were removed by a magnet. This step rendered the cell mixture essentially free of macrophages. The

remaining cells that did not phagocytose the iron particles, which included dendritic cells among others, were further processed to isolate the dendritic cells.

Song does not overcome the deficiencies in Hedley, because Song employs viral vectors. Viruses have specifically adapted to inject genetic material into cells, and one of ordinary skill in the art would not extrapolate from a virus used to introduce nucleic acids into a cell to a microparticle.

In this regard, it is respectfully pointed out that one of ordinary skill in the art (i.e., a scientist) would be acutely aware of the differences between the working examples of a patent specification (i.e., the experiments actually conducted) and remainder of the specification. In particular, one of ordinary skill in the art would read a patent specification like any other scientific publication. That is, one of ordinary skill in the art would read what experiments were actually conducted by the authors alongside other assertions by the authors regarding what might be potentially achievable and then make his or her own conclusions. Specifically, one of ordinary skill in the art would not simply assume that such other assertions are necessarily true, including (a) assertions of utility in other APC's such as dendritic cells, based on experiments with macrophages (see Hedley) or (b) assertions of utility in non-viral gene delivery vehicles, based on experiments with recombinant retroviruses (see Song). Rather, one of ordinary skill in the art would critically evaluate such statements in view of the experiments in the specification and the rest of the art.

In addition, Hedley describes microparticles with *internal* nucleic acids, rather than microparticles having *adsorbed* antigen-encoding polynucleotide as claimed in claim 54. See, for example, Hedley at col. 1, lines 32-37 ("microparticles *containing* nucleic acids"), *Id.* at col. 9, lines 2-4 ("microparticles can be prepared which carry … DNA … *within* each microparticle"), *Id*. at col. 13, lines 64-66 ("the protein or peptide encoded by the nucleic acid contained *within* the microparticle"), *Id.* at Table 5 ("[p]hagocytosis of *encapsulated* DNA leads to expression of a luciferase reporter gene construct") and Table 6 ("[e]xpression of *encapsulated* luciferase DNA in murine muscles"). (Emphasis added.)

With respect to the distinction between encapsulation vs. adsorption, the Examiner has argued that the claims *encompass* microparticles with encapsulated nucleic acid and points specifically to claims 46 and 50 in which at least a portion of said polynucleotide is entrapped within said microparticles. Office Action mailed September 22, 2004, at page 8. However, the

test for obviousness is based on whether or not there is some reason to combine reference teachings and arrive at the claimed invention, not whether or not the claims might encompass certain features of the prior art. Moreover, new claims 55 and 58 specifically exclude entrapment.

The Examiner has also alleged that "the interaction of the polynucleotide with the microparticle depends on the charge characteristics of the microparticle itself and the presence or absence of additional molecules such as detergents or surfactants. The microparticles of Hedley are not positively charged, thus combining the microparticles with the polynucleotide results primarily in encapsulation. On the other hand, Fattal clearly teaches that adding a cationic detergent to the biodegradable microparticles results in particles with a positive charge such that the majority of the negatively charged polynucleotide adsorbs onto the cationic surface rather than encapsulating therein." *Id.* at pages 8-9. With respect to the cationic detergent, the Examiner alleges that Fattal provides motivation for including a cationic detergent in a microparticle by teaching that inclusion of a cationic detergent in microparticles increases the amount of polynucleotide associated with the polymer particles and increase the uptake of the nucleic acid by phagocytosis. *Id.* at page 8.

Applicant respectfully disagrees. The polynucleotide of Hedley is encapsulated due to the double (w/o/w) emulsion particle formation process that is performed in Hedley. See col. 14, lines 22-39. The polynucleotide of Hedley is never "combined with" preexisting microparticles. In a completely non-analogous process, Fattal adsorbs a 15-mer antisense oligonucleotide onto nanoparticles by adding cationic detergent and oligonucelotides to a nanoparticle suspension in the presence of NaCl. Fattal at page 138, col. 2.

Concerning the use of cationic detergent, it is noted that Fattal observes that the poor yield of oligonucleotide association in the absence of CTAB may be explained by the fact that polyalkylcyanoacrylate (PACA) nanoparticles bear negative charges that results in electrostatic repulsion with the polyanionic oligonucleotides (page 139, col. 1), which suggests that similar results would not be obtained with neutral biodegradable polymers such as PLGA.

With regard to the Examiner's statement that including a cationic detergent in a microparticle increases the uptake of the nucleic acid by phagocytosis, Applicant wishes to clarify that uptake was said to be increased because the oligonucleotide was associated with the particles and could be phagocytosed. See Fattal at p. 138. Fattal doesn't teach, however, that

particles with associated cationic detergent are phagocytosed to a greater degree than particles without cationic detergent.

It is respectfully submitted that one of ordinary skill in the art would not have been motivated to draw inferences between the teachings of Song, Hedley and Fattal as urged by the Examiner, because these references each describes a different approach to nucleic acid delivery. For example, Song describes viral techniques as well as non-viral techniques in which polynucleotides are associated with condensing agents or encapsulated in liposomes. Song is silent with respect to microparticles. Hedley describes encapsulation of polynucleotides within microparticles, whereas Fattal teaches adsorption of oligonucleotides onto nanoparticles. With respect to the latter two techniques, at the time of the present invention, DNA adsorption and DNA encapsulation were understood by those of ordinary skill in the art to constitute separate and distinct delivery approaches, with some favoring encapsulation based on the notion that the DNA would be protected from the destructive elements (e.g., nucleases) encountered in the biological milieu, and others favoring adsorption based on the notion that the DNA would be protected from destructive elements (e.g., high shear stresses) encountered in the processing environment. By avoiding an adverse affect on nucleic acid integrity during the encapsulation process (see, e.g., Hedley at col. 14, lines 22-39), both objectives were achieved by Hedley, removing motivation to resort to the teachings of Fattal.

Moreover, Fattal merely reports the internalization of a 15-mer *oligonucleotide* (oligomer) adsorbed onto nanoparticles, and that the oligomer remains intact for several hours after cell uptake. See, e.g., Fattal Abstract and p. 140, col. 2. Clearly, oligonucleotides *per se* do not function in the same manner as polynucleotides, such as those described in Song and Hedley, which encode and express a polypeptide. Thus, the mere fact that a 15-mer oligonucleotide *remains intact* upon internalization would not have lead to a reasonable expectation that full length nucleic acid vectors such as those described in Song and Hedley would be *expressed*, as this requires, *inter alia*, delivery of a full length polynucleotide to the nucleus.

Indeed, the results shown by Fattal with regard to the 15-mer oligonucleotide are not encouraging in this respect. Specifically, Fattal teaches at page 140 that "[a]bout 20% of the oligonucleotide given *free* ***or*** *delivered by* PIHCA *nanoparticles* were found in the *nuclear* fraction." (Emphasis added.) Upon reading this, one of ordinary skill in the art would not have

been motivated to go to the effort of adsorbing antigen-expressing polynucleotides to cationic particles, because this effort would *not* have been expected to enhance the delivery of the polynucleotide to the nucleus (which is required for expression to take place in the cell) vis-à-vis the simple administration of a free polynucleotide.

Due the notable differences between Hedley and Fattal (e.g., encapsulation vs. adsorption, microparticles vs. nanoparticles, charged PACA polymer vs. uncharged PLGA polymer, antigen-encoding polynucleotide vs. 15-mer oligonucleotide, etc.), it is respectfully submitted that Fattal would not motivate one of ordinary skill in the art to include cationic detergent like that described in Fattal in conjunction with a microparticle-based transfection agent like that of Hedley, as alleged by the Examiner.

Moreover, even assuming solely for the sake of argument that one of ordinary skill in the art would be motivated to combine Fattal with Hedley to permit one to deliver more polynucleotide as urged by the Examiner, there would have been no reasonable expectation of success, given that Fattal reports no difference in nuclear-fraction oligonucleotide content between oligonucleotide given free or that delivered by nanoparticles.

Nor would there have been a reasonable expectation of success, based on the type of cells that were investigated in Hedely and Fattal. In this regard, Hedley describes administration to macrophages, whereas Fattal teaches administration to U937 cells (commonly referred to in the art as "monocyte-like" or "macrophage-like" cells). As previously indicated, one of ordinary skill in the art would immediately recognize that particulate uptake by macrophage-like cells and macrophages, which are aggressively phagocytic, would not be predictive of particulate uptake by dendritic cells, which are not.

On the other hand, one of ordinary skill in the art would have found various reasons to *avoid* the use of a cationic detergent. For example, detergents are typically added to stabilize emulsions that are commonly used to prepare microparticles and/or to impart desirable physical properties to the finished microparticle powder preparation, for example, the ability to flow freely. Nonionic detergents, in particular, polyvinyl alcohol are commonly used for this purpose (see, e.g., Example 1 of Hedley). Charged detergents, on the other hand, are less desirable, because they impart undesirable properties such as stickiness to the resulting microparticles. For this reason, one of ordinary skill in the art would have been motivated to avoid the use of cationic detergents such as CTAB. The motivation to avoid cationic detergents would have been

reinforced by the fact that nonionic detergents, such as polyvinyl alcohol, are generally known to have reduced toxicity as compared to cationic detergents, such as CTAB.

In this regard, "the Examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor and *with no knowledge of the claimed invention*, would select the elements from the cited prior art references for combination in the manner claimed." *In re Rouffet*, 149 F.3d 1350, 47 U.S.P.Q.2d 1453, 1458 (Fed. Cir. 1998). (Emphasis added.) As seen from the above discussion, this would not occur.

Accordingly, it is respectfully submitted that one of ordinary skill in the art at the time of the invention, upon considering Song, Hedley and Fattal *as a whole*, would not have been motivated to provide a method like that claimed in claim 54, nor would there have been a reasonable expectation or success, absent the hindsight gained from Applicant's disclosure.

It is therefore respectfully requested that the rejection of claim 54 in view of Song, Hedley and Fattal be withdrawn.

**Rejection of Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53**

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 are also rejected under 35 U.S.C. 103(a) as obvious over Song in view of Hedley and Fattal. This rejection is respectfully traversed for the reasons set forth below.

Claim 1, like claim 54 above, is directed to a method of transfecting dendritic cells. The method comprises incubating dendritic cells and a transfection agent that comprises a polynucleotide (which encodes an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor) adsorbed on surfaces of microparticles. The incubation is performed *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen. Thus, claim 1 is patentable over Song, Hedley and Fattal for the reasons discussed above with respect to claim 54.

Moreover, claim 1 is directed to a process that, *inter alia*, comprises incubating the dendritic cells with a transfection agent comprising an antigen-encoding polynucleotide adsorbed on surfaces of microparticles, wherein the transfection agent is formed by a process that comprises: (a) providing microparticles that comprise a biodegradable polymer and a cationic detergent, and (b) exposing said microparticles to a polynucleotide.

As previously noted, Song and Hedley are silent concerning polynucleotide adsorption, and they are also silent regarding cationic detergents. In Fattal, on the other hand, a cationic detergent (CTAB) and a 15-mer oligonucleotide are simply added to nanoparticles in suspension. See, e.g., Fattal, p. 138, col. 2. In other words, this process cannot be said to teach or suggest one in which particles that comprise a biodegradable polymer and a cationic detergent are provided and then exposed to a polynucleotide in order to adsorb the polynucleotide to the particles.

The Examiner urges, inter alia, that "[d]ue to the dynamic process of the association of the cationic detergents with the suspended microparticles, at least some portion of the particles taught by Fattal comprises CTAB before they further associate with the polynucleotide." Fattal, however, does not teach or suggest that this is the case.

Accordingly, it is respectfully submitted that a *prima facie* case of obviousness has not been established with respect to the presently pending claim 1.

Consequently, it is respectfully requested that the rejection of claim 1, and claims 2-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 depending therefrom, be withdrawn.

**Rejection of Claims 19-23**

Claims 19-23 are rejected under 35 U.S.C. 103(a) as obvious over Song in view of Hedley and Fattal. This rejection is clearly respectfully traversed.

First, claims 19-23 depend from claim 1 and are thus patentable over Song, Hedley and Fattal for the reasons set forth in the prior sections.

Moreover, it is noted that claims 19-23 are directed to a procedure in which dendritic cells are transfected *ex vivo* in accordance with claim 1, and then administered to a vertebrate subject in an amount effective to produce an immune response.

As noted above, Song teaches nothing about microparticles as delivery vehicles and Song expresses a clear preference for direct injection of recombinant retroviruses (see, e.g., Song, page 27, lines 25-27) over the use of *ex vivo* techniques.

As noted above, Hedley does teach that microparticles can be introduced intradermally (i.e., to the APCs of the skin, such as dendritic cells and Langerhans cells). Unlike the claimed method, however, this single mention of dendritic cells in Hedley is in conjunction with an *in*

*vivo* technique. The remainder of Hedley is directed to macrophages, which are fundamentally different from dendritic cells as noted above.

The Examiner has referred to column 12 and Example 2 of Hedley as supporting *ex vivo* techniques. At col. 12, lines 23-30, Hedley refers to "in vitro/ex vivo use." However, that use is clearly experimental, as opposed to therapeutic. Hedley states nothing about administering dendritic cells that have been transfected *ex vivo* to a vertebrate subject. Instead, Hedley merely states that "[t]he [mammalian] cells can be either analyzed immediately or recultured for future analysis.") *Id.* Similarly, while Example 2 of Hedley describes an *in vitro* cell study, this is just a prelude to the *in vivo* cell studies in Examples 3 *et seq.* to follow. An *in vitro* cell study with macrophages is far removed from a procedure in which dendritic cells are transfected *ex vivo* in accordance with claim 1, and then administered to a vertebrate subject in an amount effective to produce an immune response, as claimed in claims 19-23.

Indeed, from a therapeutic standpoint, Hedley as a whole is clearly directed to *in vivo* treatment techniques. In this regard, Hedley teaches various methods for *in vivo* delivery including (a) direct delivery into the bloodstream (i.e., by intravenous or intraarterial injection or infusion), (b) subcutaneous injection, (c) intradermal delivery, (d) delivery via the gastrointestinal tract and (e) introduction of microparticles into the lungs. *Id.* at col. 8, lines 20-34. See also col. 13, lines 15-20. All in vitro teachings of Hedley are related to experimental, rather than therapeutic procedures.

Fattal, like Hedley, teaches nothing about administering dendritic cells (or any other cells) that have been transfected *ex vivo* to a vertebrate subject.

Accordingly, it is respectfully submitted that a *prima facie* case of obviousness has not been established with respect to presently pending claims 19-23.

Consequently, it is respectfully requested that the rejection of claims 19-23 be withdrawn.

**CONCLUSION**

It is respectfully submitted that all claims are presently in condition for allowance. Should the Examiner be of the view that an interview would expedite consideration of the application, request is made that the Examiner telephone the Applicants' attorney at (703) 433-0510 in order that any outstanding issues be resolved.

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